Allyl Thiocyanate – Natural Product or Artifact in Crucifer Extracts?

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Key Words
Gas chromatography
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Summary
Allyl thiocyanate and isothiocyanate were synthesized and could be analysed by gas chromatography without isomerization by keeping the injector at, or below, 50 °C. Synthetic allyl isothiocyanate contained none of the isomeric thiocyanate but allyl thiocyanate contained 0.5 % of isothiocyanate. The NMR spectra indicated both compounds to be free of the corresponding isomer. Analysis of extracts of stinkweed, mustard and horseradish by GC using non-isomerizing conditions showed that only the stinkweed extract contained allyl thiocyanate. Mustard and horseradish extracts contained allyl, and other, isothiocyanates but no allyl thiocyanate. Allyl thiocyanate previously reported in extracts of mustard and horseradish was most likely an artifact caused by partial isomerization of allyl isothiocyanate under the conditions of isolation and/or analysis.

Introduction
In a recent paper [1], headspace volatiles collected from Japanese horseradish (Wasabi japonica) at 90 °C were found to contain allyl isothiocyanate and the corresponding thiocyanate. It was suggested [1] that the thiocyanate arose from reversible isomerization of the isothiocyanate on the chromatography column. However, Gilbert and Nursten [2], who also observed these products in extracts of Japanese, English and Hungarian horseradish, reported that allyl isothiocyanate, isolated by preparative gas chromatography, did not isomerize when rechromatographed.

Thiocyanates as products of the enzymatic hydrolysis of glucosinolates are relatively rare. Benzyl thiocyanate was obtained by autolysis of seeds of Lepidium spp. (garden cress) [3] and 3-methylthiobutyl thiocyanate from Eruca sativa (salad rocket) [4]. Allyl thiocyanate was identified as the main product of autolysis of Thlaspi arvense (stinkweed, penny-cress) [3] and was shown [5] to be the initial product which subsequently isomerized to allyl isothiocyanate (1).

\[ \text{CH}_2 = \text{CH} - \text{CH}_2 - \text{SCN} \leftrightarrow \text{CH}_2 = \text{CH} - \text{CH}_2 \text{NCS} (1) \]

These results together with the demonstration of a “thiocyanate-forming factor” [6], and the observation that allyl isothiocyanate did not isomerize during gas chromatography [2], suggest that the allyl thiocyanate isolated from horseradish [1, 2] could be a natural product and not a laboratory artifact.

The flavours of crucifers and associated condiments are attributed [7] mainly to the various isothiocyanates released when endogenous glucosinolates are hydrolysed by native myrosinase. Allyl isothiocyanate is described as pungent, lachrymatory [2, 7] and bitter [7]. The effect of thiocyanates on flavour does not appear to have been studied [7] although MacLeod [7] suggested that besides adding a garlic taste, decomposition to thiols could introduce a foetid character.

In this laboratory, interest in crop-pest interaction led to a study of the attraction of diamondback moths (Plutella xylostella) to volatiles from macerated oriental mustard (Brassica juncea, c.v. Domo) [8]. The main component of the volatiles, allyl isothiocyanate, was accompanied by a small amount of allyl thiocyanate [9]. The latter compound has also been found in the oil obtained by steam distillation of oriental mustard seed [10].

As a result of these observations, and the possibility that allyl thiocyanate could influence the attraction of pests or the flavour of condiments, the synthesis and gas chromatography of allyl thiocyanate and isothiocyanate were studied. In addition, extracts of plants reported to give allyl thiocyanate and isothiocyanate were also examined.
Experimental

Sample Preparation

Seeds of *Thlaspi arvense* (0.5 g) were ground in hexane, washed with pentane to remove oils and air dried. For autolysis the meal was made into a paste with water at room temperature (25 °C) and diluted to 5 ml in a test tube (15 x 1.5 cm). Hexane (2 ml) was added and the mixture stirred magnetically to induce a slight vortex in the solvent layer. Samples of the hexane were removed periodically, placed in Dry Ice for 5 min to solidify any water and stored at -15 °C until analyzed. The procedure was repeated using cold (0 °C) water and hexane with ice cooling in a cold room (4 °C) to minimize risk of isomerization.

The above procedures were applied also to Keen’s mustard (Colman Foods, Norwich, England) while seeds of oriental mustard were treated only at room temperature. Horseradish root (*Armoracia* spp. Van Bloem’s, Holland Bulb Co., Portland, Oregon, USA) was homogenized in water (20 ml/g) at room temperature.

Synthesis of Allyl Isothiocyanate

Allyl amine (1.14 g, 0.02 mole) in dichloromethane (5 ml) was added over 10 min to thiocarbonyldimidazole (3.56 g, 0.02 mole, Aldrich Chem Co., Milwaukee, USA) in solvent at 0 °C [11]. The mixture was stirred at 0 °C for a further hour and for 1 hr at room temperature. The solvent was removed at room temperature under vacuum and the residual solid extracted repeatedly with pentane to give a light yellow oil (0.85 g, 43%). The NMR spectrum in CDCl₃ (Figure 1a) showed a multiplet at δ 4.12 ppm (Lit. [12] 4.18 ppm) corresponding to the α-protons with no indication of signals at higher field due to thiocyanate (cf. Figure 1b).

Synthesis of Allyl Thiocyanate

Synthesis was performed using a slight modification of the method of Emerson [13]. A mixture of allyl bromide (3.6 g, 0.03 mole) in ethanol (95 %, 10 ml) and sodium thiosulfate pentahydrate (7.44 g, 0.03 mole) in water (10 ml) was heated on the steam bath for 1 hr. After cooling to room temperature, the mixture was extracted with ether to remove unreacted bromide and allyl sulfides. The aqueous residue was cooled in ice and powdered KCN (1.95 g, 0.03 mole) added with stirring over 15 min. After a further 20 min the cold mixture was repeatedly extracted with pentane, the combined extracts dried (Na₂SO₄) and evaporated to dryness at 0 °C to give a pale yellow liquid (2.56 g, 86 %). Analysis by NMR (CDCl₃) at 0 °C showed a doublet at δ 3.52 ppm (Figure 1b) corresponding to the α-protons. There were no signals at δ 4.12 ppm indicative of allyl isothiocyanate (cf. Figure 1a).

Gas Chromatography

Samples were analyzed by splitless and on-column injection onto a 30 m x 0.32 mm i.d. DB-23 column (0.25 μm, J&W Scientific, Folsom, CA, USA) mounted in a Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) equipped with FID, split-splitless and on-column injectors. Helium at 2.3 ml/min was used as carrier gas. The detector was kept at 260 °C and the injector varied as in Table I. The oven was programmed at 50 °C/5 min, 4 °C/min to 80 °C, 15 °C/min to 240 °C. When on-column injections were made, a 1 m x 0.53 mm i.d. deactivated fused silica retention gap (Chrompack, Middelburg, The Netherlands) was